1   Fall composition of storage lipids is associated to the overwintering strategy of *Daphnia*

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29   **Abstract**

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31   Diapause, using dormant eggs, is a strategy some zooplankton use to avoid winter

32   months of persistent low temperatures and low food availability. However, reports of

33   active zooplankton under the ice indicate other strategies also exist. This study aimed at

34   evaluating whether fall composition of storage lipids is different between diapausing and

35   actively overwintering *Daphnia*. We assessed the quantity of storage lipids and fatty acid

36   composition of *Daphnia* species along with fatty acid content of seston in six boreal,

37   alpine and subarctic lakes at the onset of winter, and evaluated the association of storage

38   lipids to *Daphnia* overwintering strategy. We found that active overwintering *Daphnia* had

39   >55% of body fat and the highest fatty acid concentrations. Polyunsaturated fatty acids,

40   especially stearidonic acid (18:4n3; SDA) and high ratios of n3:n6 were preferentially

41   retained in active overwintering *Daphnia* compared to those that entered diapause. The

42   *Daphnia* fatty acid compositions were independent of that of the seston diet, indicating

43   *Daphnia* adjusted their storage lipids according to the physiological requirements of a

44   given overwintering strategy. The occurrence of the active overwintering strategy has

45   consequences to zooplankton community structure, and can have important implications

46   for the transfer of high quality energy in higher trophic levels.

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48   **Keywords:** freshwater, winter, fatty acids, ice, life history strategies, zooplankton, SDA

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55 **Abbreviations**

56 FA Fatty acid(s)

57 FAME Fatty acid methyl esters

58 EPA Eicosapentaenoic, 20:5n3

59 MUFA Monounsaturated fatty acid(s)

60 PUFA Polyunsaturated fatty acid(s)

61 SDA Stearidonic acid, 18:4n3

62 SFA Saturated fatty acid(s)

63 **Introduction**

64

65   Life history strategies of organisms are often determined by environmental cues, such as

66   temperature and food availability [1–3]. This means that organisms can adjust their

67   physiology and reproductive cycles to changes in their environment. One of the best

68   documented life-history adaptations to adverse changes in the environment is the

69   production of resting eggs, ephippia, in *Daphnia* (Cladocera) [4, 5]. Increases in

70   population density and decreases in temperature, light and food supply are some of the

71   most common triggers that induce resting egg production, and characterize lakes in late

72   summer – fall. It is therefore commonly accepted that cladocerans prepare for winter by

73   producing ephippia and are absent from the water column during the winter months with

74   persistent low temperatures and limited food. However, there is increasing awareness of

75   the presence of active zooplankton throughout the ice cover period [6–8], indicating that

76   at least for certain species there exist also other adaptation mechanisms to overwinter.

77   While most of the historic research has focused on environmental conditions initiating

78   overwintering diapause, especially ephippia production in *Daphnia* [9], much less is

79   known about physiological adaptations of cladocerans that overwinter actively.

80

81   An effective physiological overwintering mechanism known for fish and marine

82   zooplankton is to accumulate storage fat [5, 10, 11]. Storage fats and their fatty acids

83   (FA) provide high-density energy reserves used to maintain metabolic function and

84   reproduction during low food supply [12–14]. Studies on freshwater copepods indicate

85   that both the quantity of lipid reserves [15] and the high overall percentage of

86   polyunsaturated fatty acids (PUFA) [16] are common characteristics in the fall for

87   copepods that overwinter actively suggesting that there could be differences in lipid

88   accumulation depending on whether cladocerans enter diapause or overwinter actively.

89   The link between storage fats and FA, and zooplankton life history strategies has

90   however not been explored in detail, especially in freshwater systems.

91

92   Typically the function of particular fatty acids is determined by the degree of saturation,

93   and fatty acids can be grouped accordingly. Saturated and monounsaturated fatty acids

94   (SFA and MUFA) tend to be used for storage fats [17]. PUFA are particularly important

95   for reproduction and early development [17], and are used to compensate for the loss of

96   cell membrane flexibility in cold temperatures [18]. Further, consumers differ in their

97   composition and thus in their requirement for specific FA [19]. The growth and

98   reproduction of cladocerans has e.g. been strongly affected by the EPA

99   (eicosapentaenoic, 20:5n3) content [20] but whether EPA or any other FA is linked or

100   required to the active overwintering *Daphnia* is not known. Little is also known about how

101   composition or relative differences between certain FAs account for any species specific

102   or individual metabolic requirement, such as an organism's reproduction, thermal

103   compensation, or life-history stage. Generally a higher quantity of PUFA and a higher

104   ratio between omega-3 (n3) and omega-6 (n6) PUFA are favorable for all organisms,

105   however the optimum dietary ratio of n3:n6 seems to be highly species specific [21] and

106   very little is known about changes in seasonal requirements for these FA ratios.

107

108   As the source of zooplankton lipids is diet, any seasonal accumulation of lipids in

109   zooplankton is linked to food sources. Seasonal phytoplankton food availability has been

110   reported to be the main driver for organisms to store FA [3, 17] and there is evidence that

111   freshwater copepods use fall phytoplankton to build up the fats that allow for reproduction

112   under the ice [8, 16]. Changes in phytoplankton community and FA composition are to

113   some extent mirrored in the zooplankton FA composition [22, 23]. However, in fall, a

114   simple reflection of the bulk phytoplankton community, where many taxa may be

115   senescing, would not be a winning strategy to survive the winter months under the ice.

116   Rather, a selective accumulation of FAs could be used as an energy source during

117   winter. Given the predominance of ice cover in northern latitudes, it is important to

118   understand the environmental and physiological mechanisms determining life history

119   strategies. Ultimately the presence or absence of an active zooplankton in a lake affects

120   the lake's community structure and ecological functioning, such as winter food web

121   dynamics [24, 25].

122

123   In this study we investigate the link between winter life history strategy of *Daphnia* and

124   the quantity of their lipids and FA composition. *Daphnia* are well adapted to unstable

125   habitats due to short life cycle, and have a wide phenotypic plasticity [6, 26]. These

126   characteristics make *Daphnia* an ideal genus to compare the physiology and

127   overwintering strategies among different populations in different parts of their

128   environmental range. Our primary goal of this study was to compare the FA composition

129   of *Daphnia* from boreal, alpine and subarctic lakes at the onset of winter. In this wide

130   geographical range, different species compositions of *Daphnia* and different

131   overwintering strategies emerged, including *Daphnia* that entered diapause for winter,

132   *Daphnia* that remained active under the ice, and lakes where both overwintering

133   strategies were present. We hypothesized that in lakes where *Daphnia* overwinter

134   actively, they will store lipids during the fall, particularly PUFA, in order to compensate for

135   low temperature and limited food availability under the ice. Since the dormant strategy

136   simply avoids the harsh winter conditions, we assumed that *Daphnia,* which enter

137   dormancy will not invest in storing lipids. We include further discussions on the

138   evolutionary implications of winter strategies, and the composition and physiology of

139   storage lipids.

140

141   **Materials and methods**

142

143   *Study sites and sample collection*

144   We sampled *Daphnia* from six lakes across three geographically distinct regions in 2009-

145   2011 (Table 1). The *Daphnia* were the dominant zooplankton species in three of the sites

146   (Big Rock, Malla South and Mekkojärvi) while in the other sites they contributed to a

147   zooplankton community dominated by copepods. Due to the wide geographical range the

148   dataset included five different *Daphnia* species and many lakes had different *Daphnia*

149   assemblages (Table 1). In the text they are collectively referred to as *Daphnia*. The lakes

150   were divided to three categories based on the occurrence of *Daphnia* in winter. The lakes

151   Malla South and Saanajärvi with no record of ephippia-carrying *Daphnia* in earlier

152   zooplankton samples or paleolimnological studies (e.g. [27]) were considered lakes with

153   actively overwintering *Daphnia*. The lakes where all *Daphnia* entered diapause as

154   ephippia due to freezing solid (Big Rock) or winter anoxia (Mekkojärvi) were called

155   diapause lakes. The lakes Lunzersee and Simoncouche, where both ephippia and active

156   individuals were present, were considered lakes with both overwintering strategies.

157   *Daphnia* were present in winter in the alpine Lunzersee (0.0005 ind L^{-1}, n=1, December)

158   with the shortest ice cover period of three months, in boreal Simoncouche (2.46±5.5 ind

159   L^{-1}, n=12, December-April) with six months of ice and the two subarctic lakes Malla South

160   and Saanajärvi (0.02±0.01 ind L^{-1}, n=8, November-May) with up to nine months of ice.

161   The winter abundance was up to two fold lower than in summer, however,

162   parthenogenetically reproducing individuals were found in both lakes in winter.

163

164 From these six sites we compared the percent body fat and FA composition of adult

165 *Daphnia* at the onset of winter. Considering the wide latitudinal variation in our study sites

166 this period was defined as maximum the two months before ice cover for each water

167 body (hereafter referred to as fall). Temperature in all sites was close to 10°C during the

168 sampling and decreasing from the maximum summer values. *Daphnia* were collected by

169 vertical tows of a 50 μm mesh net at the deepest point of the lake site, or by horizontal

170 pulls in the shallow pond Big Rock. The *Daphnia* were hand sorted, aiming for at least

171 0.5-1.5 mg dry weight (DW), when possible triplicates were made. Seston, representing

172 the dietary source of FA available for *Daphnia*, was taken from a depth-integrated water

173 sample that was pre-filtered through a 50 μm net to remove larger organisms, then

174 filtered onto a GF/F filter using 1-3 L per triplicate. All samples were freeze dried and

175 stored in -80 °C until lipid and FA analysis.

176

177 *Fatty acid analyses*

178 Fatty acids were analysed using a three-step extraction-methylation protocol [27]. Freeze

179 dried and weighed *Daphnia* and seston samples were extracted using a chloroform-

180 methanol wash cycle and concentration of extracted lipids determined by gravimetry. The

181 body fat percentage was calculated as a function of extracted lipid weight and *Daphnia*

182 DW. The extracted lipids were methylated using toluene and sulphuric acid-methanol and

183 then solubilized in hexane. Fatty acids (C14–C24) were identified as fatty acid methyl

184 esters (FAME) using a gas chromatograph equipped with a Supelco™ SP-2560 column

185 (100 m, 25 mm i.d., 0.2 μm film thickness) and a flame ionization detector (TRACE GC-

186 FID THERMO™). Total fatty acid concentrations (ΣFAME) were calculated using

187 calibration curves based on known external standard concentrations and are reported as

188 μg FAME per mg carbon weight (μg FA mgC^{-1}). Fatty acid concentrations were calculated

189 using calibration curves of known standard concentrations. *Daphnia* dry weights were

190 converted to carbon (C) weight using a *Daphnia* summer average of 42% C and 15% for

191 seston that were available from mass spectrometry (Thermo Finnigan DELTAplus

192 Advantage) and stable isotope analyses (Mariash unpubl). Only the FAME greater than

193 0.1% were used in the analysis. All FAME results are presented as a proportion of the

194 total FAME concentration in moles. Individual FA are summarized in results into general

195 lipid classes of saturated- (SFA), monounsaturated- (MUFA), polyunsaturated- (PUFA)

196 fatty acids or as ratios of the sum of omega- 3 to omega-6 FAME (n3:n6).

197

198 *Statistical analysis*

199 Differences in seston and *Daphnia* fatty acid composition (data normalized, Euclidean

200 distances), and *Daphnia* fall FAME characteristics (ΣFAME, %PUFA, %SFA, %SDA,

201 SFA:PUFA and Σn3:n6) and body fat levels among strategies (active, diapause, both

202 strategies) and lakes (Malla South, Saanajärvi, Simoncouche, Lunzersee, Big Rock,

203 Mekkojärvi) were tested with a 2 factor (lake nested in strategy, strategy fixed and lake

204 random) Permutational (Multivariate for fatty acid compositions) Analysis of Variance

205 (PERMANOVA with 999 permutations). While comparing FAME characteristic values can

206 be done with classical ANOVA, permutational ANOVA was preferred as it did not require

207 observations fitting the normality assumptions (see [29]). The nested design was used to

208 control for variation among lakes within strategy. When only a restricted number of

209 permutations were possible, Monte Carlo *p*-values were used. Non-metric multi-

210 dimensional scaling (nMDS) was used to visualize *Daphnia* FA composition among

211 factors. Linear regressions were carried out between *Daphnia* and seston FAME

212 variables that were significantly different in *Daphnia* in different overwintering strategies

213 (ΣFAME, %PUFA, %SDA, Σn3:n6) to test for potential diet influence on *Daphnia* FAME

214 composition. Pair-wise comparison tests among strategies were carried out for *Daphnia*

215 FAME composition, total FAME, PUFA, SDA and n3:n6 in PERMANOVA with adjusted

216 significance level method from Holm [30]. All analyses were conducted using

217 PRIMER+PERMANOVA version 7.0.9. A significance level *α* = 0.05 was used for all

218 statistical tests.

219

220 **Results**

221

222 The *Daphnia* total lipid amount and FA profiles were different for *Daphnia* with different

223 strategies (Table 2, Table 3, Fig. 1). In the lakes with actively overwintering *Daphnia*, the

224 percent body fat was highest (58%) although not statistically different from the other

225 strategies (Table 3a). In the lakes with diapause or mixed overwintering strategy *Daphnia*

226 had lower body fat (~30%) (Table 2a). Total FAME concentrations (mean ±SD) were

227 statistically higher for active *Daphnia*, than for the diapause strategy (Table 2b, Table 3b,

228 Fig. 2). The *Daphnia* FA compositions among the strategies were marginally different (p =

229 0.068) from each other (Table 3c; Fig 1). When the lake factor was not considered all

230 strategies were highly statistically different from each other (p < 0.003 for all multiple pair-

231 wise comparisons) and sample scores from the active and diapausing populations were

232   furthest away from each other (Fig. 1). The difference among differently overwintering

233   *Daphnia* FA compositions was largely due to differences in their total PUFA, stearidonic

234   acid (18:4n3; SDA) and the n3:n6 ratio (Fig. 2). The *Daphnia* PUFA were marginally

235   different between strategies (p = 0.061; Table 3d) with higher values in actively

236   overwintering *Daphnia* (Table 2c). SDA was significantly different among strategies

237   (Table 3f, Fig. 2) and more than double in active winter *Daphnia* (26% of total FAME)

238   compared to *Daphnia* with mixed strategies (12%) and 9-fold higher compared to the

239   diapausing *Daphnia* (4%) (Table 2c). The SDA results were further reflected in the n3:n6

240   differences among strategies with significantly higher n3:n6 ratios for the active strategy

241   (Table 2d, Table 3h). In summary, the main lipid parameters that were most different

242   between strategies were total FAME concentration, SDA, PUFA, and n3:n6 FA ratio, as

243   illustrated in Figure 2. Further, the active and diapausing populations differed in species.

244   Lakes with active strategy had only *D. umbra* and lakes with diapause had only *D.*

245   *longispina,* which differed in their FA composition (SI Table 1).

246

247   FAME concentrations in seston were an order of magnitude lower than *Daphnia* FAME

248   concentrations (SI Table 2). Seston had high SFA:PUFA ratios, indicating that SFA

249   dominated the seston. Of all FAME in seston, between 41 to 89% were SFA. Of the

250   PUFA, seston generally contained very small amounts of 18:3n6 (GLA), 20:5n3 (EPA),

251   22:6n3 (DHA), and notably 18:4n3 (SDA) was less than 2%. Seston FA composition was

252   not different among strategies (Table 3i). Regression analyses revealed that variation in

253   seston FA composition (ΣFAME, %PUFA, %SDA, Σn3:n6) across the six lakes did not

254   explain a significant amount of corresponding variation in the FA composition of any

255   *Daphnia* community (R^2 < 0.20 and p > 0.10 for all comparisons). While average

256   concentrations of individual FAs in the *Daphnia* varied substantially among overwintering

257   strategies, FA concentrations in seston were more uniform and did not reflect these

258   differences. The patterns in FA composition and total fatty acid concentrations in the

259   zooplankton appeared more closely related to zooplankton overwintering strategy than

260   seston fatty acid composition.

261

262   **Discussion**

263

264   We compared the quantity of storage lipids and FA composition of *Daphnia* at the onset

265   of winter from lakes where there is an active winter community, lakes where the *Daphnia*

266 enter diapause for winter and from lakes where the *Daphnia* use both strategies but are

267 dominated by the diapausing strategy. Our results suggest that fatty acids may have an

268 important role for *Daphnia* that prepare to overwinter actively and are in accordance with

269 earlier observations from cladocerans and calanoid copepods that have demonstrated a

270 high accumulation of lipids and PUFA in fall [5, 15, 16]. Storing fat could be a

271 physiological mechanism zooplankton use to adapt to life under the ice, and therefore

272 can potentially be used as a physiological indicator for zooplankton's winter survival.

273

274 The active winter *Daphnia* stored more FA, both in % body fat and FAME concentration,

275 than the other two strategies. Specifically PUFA, SDA (18:4n3), and high ratios of n3:n6

276 were preferentially retained in active overwintering *Daphnia* than in *Daphnia* using the

277 other two strategies. While cladocerans should have fairly constant n3:n6 FA ratios, with

278 an average of 3 [19, 31, 32], our results show that there were contrasting n3:n6 ratios

279 between winter life history strategies. The active *Daphnia*, especially in the subarctic, had

280 more than double (8.25) the average, while the diapausing *Daphnia* were below (2.0) the

281 n3:n6 average value of three. High proportions of SDA mostly drive the n3:n6 ratios in our

282 results, highlighting that SDA could have an important role in physiological functions for

283 *Daphnia* winter life history strategy.

284

285 We also considered the alternative explanation that the patterns in FA among *Daphnia*

286 may stem from differences in basal resources [33] rather than the overwintering strategy.

287 For example, changes in FA among *Daphnia* in boreal lakes were attributed to changes

288 in resource assimilation between summer and fall diets [34]. However, our results show

289 that the composition of the *Daphnia* FA indicated a preferential retention or accumulation

290 of specific FA by *Daphnia*, rather than a direct reflection of availability of specific FA in

291 seston during the fall. Further, as winter seston diet is more scarce in subarctic than in

292 boreal lakes, one would expect diapause to be the dominant strategy at high-latitude

293 lakes, which was not the case here. The *Daphnia* entered diapause only when

294 environmental constraints such as loss of habitat (drying, freezing solid, anoxia)

295 prevented the active stage. While the limited number of lakes in this study does not allow

296 for more accurate testing for the region's or ice cover's role on the strategy, our data

297 shows that *Daphnia* overwinter actively in subarctic lakes even when there is 9 months of

298 ice cover.

299

300

301 Other factors known to affect life history strategies are species type [35], environmental

302 stressors like temperature [2], and/or lake size and depth [36, 37]. Our study included five

303 different *Daphnia* species and it is known that different species differ in their fatty acid

304 composition and allocation of lipids [38, 39]. Lakes with active winter *Daphnia* had only *D.*

305 *umbra, a species* that is not known to occur in temperate lakes, and both lakes with

306 diapause had only *D. longispina, a species* common in the boreal region. It is possible

307 that lakes only support populations of species that are matched to the environment in

308 terms of their physiology, including fatty acid signatures. Hence, it could be that not all

309 *Daphnia* species are able to regulate their storage fats and lipid composition to the same

310 extent. The species that do not possess this ability could be geographically restricted to

311 lakes where open water period is warm and long enough to allow for the emergence from

312 ephippium and completing a life cycle before environmental conditions turn adverse

313 again. Subsequently, species not capable for lipid accumulation should be absent from

314 perennially cold arctic lakes, which could contribute to explaining the low species number

315 of zooplankton in arctic lakes [40]. However, there exists evidence that lipid allocation is

316 not only a species-specific trait but also determined by environmental conditions. Based

317 on the number of lipid droplets in *D. umbra,* Larsson and Wathne [5] were able to

318 separate the ephippia producing individuals with less lipid droplets from individuals that

319 accumulated large amounts of lipids to survive longer. Nevertheless, regardless of

320 whether overwintering actively is an intrinsic physiological adaptation of only certain

321 species or a more common response of *Daphnia* genus to seasonally changing

322 environmental conditions, the results here are in line with other studies that have shown

323 that zooplankton which overwinter actively accumulate lipids for the subsequent use

324 during the months when diet is scarce [8].

325

326 As observed also in other studies, both strategies can coexist in the same lake [5, 36,

327 37]. Although it would be interesting to separate the different life history strategies within

328 the same lake, there is no way of visually separating between individuals that will

329 overwinter actively versus those that will make ephippia. Even at a population level, it is

330 difficult to discern the overwintering strategy. It is however, noteworthy that in lakes

331 where both strategies existed, the majority of *Daphnia* entered diapause based on the

332 low numbers of active *Daphnia* in water column under the ice and the high number of

333 ephippia in fall. The more similar fatty acid composition between diapausing and mixed

334   strategies in fall further suggests that most *Daphnia* in the mixed strategy lakes entered

335   diapause, possibly because they did not possess physiological means to accumulate

336   storage fats. FA composition between active, diapausing, and both strategies from

337   different lakes and different species, in this study suggests that lipid accumulation is an

338   important factor for *Daphnia* winter strategies, however, further experimental approach

339   and tests would need to be used to explicitly determine this.

340

341   The ecology of the subarctic and arctic region may provide a framework to understand

342   how the active winter strategy evolved. The intense light regime in late spring at higher

343   latitudes promotes a thriving phytoplankton community even under the ice [7, 41, 42],

344   creating favorable growing conditions for *Daphnia*. At this time ephippia would not yet be

345   primed for emergence as water temperatures are still too low (3 - 5°C) and would not be

346   able to benefit from the spring phytoplankton, as the already active community would.

347   Further, the high levels of the polyunsaturated SDA and n3 found in the active *Daphnia*

348   can help to combat the low spring temperatures [43]. Active winter *Daphnia* and their

349   parthenogenetic young are able to exploit the early spring phytoplankton, and are

350   typically larger in body size [44] giving them a decisive competitive advantage over the

351   resting egg community, made possible by energy reserves stored as > 55% body fat as

352   suggested by our study. In addition, the interaction between low temperature and

353   starvation during winter months could effectively help to extend winter survival by

354   lowering metabolism [45]. When the open water season is a major part of the year,

355   *Daphnia* have time to feed and regenerate within the longer productive period, and

356   ephippia have the flexibility to postpone emergence until spring conditions improve.

357

358   The evolutionary advantages of reproducing even in times of starvation are, however,

359   weighed against living longer but with increased probability of death from senescence

360   [46]. Complete abandonment of ephippia would be risky as a single season of low

361   survival and failed reproduction could eliminate an entire population. Further, the

362   diapause strategy maintains genetic diversity and ensures that a population may persist

363   during potentially fatal conditions [4]. The most profitable strategy would be to produce

364   many parthenogenetic offspring in spring plus add to the ephippia bank for insurance [1].

365   We found the two winter life histories across the regions studied although a larger dataset

366   would be needed to confirm the occurrence and potential dominance of this strategy at a

367   global scale.

368

369 The occurrence of different overwintering strategies has consequences to the plankton

370 community structure and food web dynamics. Not only is the seasonal accumulation of

371 PUFA in cladocerans important to the accumulation of quality energy to higher trophic

372 levels [47], but the actual presence of winter *Daphnia* is important to winter-feeding fish

373 ([24] and ref therein) and can affect the composition of the spring phytoplankton

374 community [48]. Our results show that *Daphnia* using the diapause strategy do not need

375 to invest in storing a large reserve of lipids, having only half as much body fat in the fall

376 compared to the *Daphnia* remaining active. Therefore both population and community-

377 level dynamics are affected by the life history strategies of *Daphnia*. Further, the

378 physiological adaptations and life history strategies zooplankton use on an annual scale

379 may provide insight into the degree of population plasticity in response to a changing

380 climate. As temperatures continue to increase around the globe, earlier spring ice off or

381 later fall ice-on will begin to affect the environmental cues (temperature and photoperiod)

382 for diapause stages [35, 49]. A longer ice-free period will change the phytoplankton

383 community and production, and could lead to changes of storage fat accumulation for

384 zooplankton.

385

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533

Tables and Figures

Table 1. Lake locations and physical characteristics separated by *Daphnia* winter strategy (active, both strategies, diapause).

	Country	Region	Latitude N	Longitude E	Altitude m	Area m²	Depth m	Ice cover months	*Daphnia* species
Active									
Malla South	Finland	subarctic	69°03	20°43	599	7850	3.0	9	*D. umbra*
Saanajärvi	Finland	subarctic	69°05	20°97	679	7.00E+05	24	8	*D. umbra*
Both strategies									
Simoncouche	Canada	boreal	48°25	-71°94	110	8.70E+05	6.0	6	*D. galeata, D. dubia, D. longiremis*
Lunzersee	Austria	alpine	47°51	15°04	608	6.80E+05	34	3	*D. longispina*
Diapause									
Big Rock	Finland	subarctic	69°10	20°43	527	47	0.5	9	*D. longispina*
Mekkojärvi	Finland	boreal	61°13	25°08	130	3800	4	6	*D. longispina*

Table 2. Average (±SD) values of *Daphnia* a) body fat (% of dry weight), b) total FAME concentration (μgFA mgC^{-1}), c) fatty acid composition (mole % of total fatty lipids) for saturated (ΣSFA), monounsaturated (ΣMUFA), and polyunsaturated (ΣPUFA) fatty acids, d) ratios of ΣSFA:PUFA and Σn3:n6 per overwintering strategy. n = number of replicates, nd = no data. Bold values are sums of the individual FAME.

	Active n=7	Both Strategies n=9	Diapause n=7
a) Body fat	58.6 ±11.0	30.9 ±3.3*	32.6 ±4.1
b) **ΣFAME**	**742.6 ±132.0**	**189.7 ±57.3**	**214.7 ±10.6**
c) **ΣSFA**	**27.0 ±0.1**	**32.1 ±6.1**	**26.5 ±5.5**
14:0	9.7 ±0.3	11.6 ±7.2	3.7 ±2.03
15:0	0.9 ±0.1	0.7 ±0.9	1.1 ±0.1
16:0	10.7 ±0.3	15.6 ±0.5	14.9 ±5.9
17:0	0.2 ±0	0.8 ±0.2	0.8 ±0.1
18:0	5.6 ±0.4	3.4 ±0.2	6.1 ±1.9
ΣMUFA	**15.4 ±0.6**	**26.4 ±2.3**	**43.0 ±16.3**
16:1n7c	5.9 ±0.1	8.9 ±0.4	25.3 ±19.8
18:1n9	5.6 ±0.4	8.5 ±0.9	8.2 ±4.8
16:1n9	1.4 ±0.1	4.0 ±3.6	0.9 ±4.7
18:1n7	2.6 ±0.2	5.0 ±2.5	8.7 ±1.1
ΣPUFA	**57.6±0.8**	**41.5 ±8.3**	**30.5 ±10.8**
18:2n6	3.5 ±0.3	4.5 ±0.3	5.0 ±2.3
18:3n3	5.2±0.2	6.9 ±2.6	6.8 ±4.8
18:4n3	25.7 ±0.4	11.6 ±1.6	3.5 ±1.1
18:3n6	1.3 ±0.1	0.8 ±0.2	0.6 ±0
20:4n3	9.0 ±1.8	0.6 ±0.1	0.6 ±0.1
20:4n6	1.6 ±0.2	3.8 ±0.1	4.7 ±1.1
20:5n3	10.6 ±0.5	12.8 ±3.6	9.0 ±4.4
22:6n3	0.8 ±0.2	0.8 ±0.1	0.4 ±0.2
d) **ΣSFA:PUFA**	**0.5 ±0.4**	**0.8 ±0.3**	**0.9 ±0.1**
Σn3:n6	**7.9 ±0.2**	**3.6 ±0.8**	**2.0 ±0.1**

*this value is only from Lake Simoncouche, Lunzersee did not have a sample for fall body fat.

Table 3. Summary of the permutational ANOVA comparing *Daphnia* a) body fat, b) total FAME, c) FA composition, d) PUFA, e) SFA, f) SDA, g) SFA:PUFA, (h) n3:n6, and i) seston FA composition between the overwintering strategies (active, diapause, both) at the onset of winter. Except for i), all *p*-values were obtained with Monte-Carlo routine (see methods).

Source	df	MS	Pseudo-*F*	*p*-value
(a)　Body fat				
Strategy	2	1318.9	4.27	0.182
Lake(strategy)	2	256.29	3.17	0.069
Residual	13	80.9		
Total	17			
(b)　Total FAME				
Strategy	2	7.08×10^5	17.4	0.023
Lake(strategy)	3	40852	4.69	0.008
Residual	17	8717		
Total	22			
(c)　FA composition				
Strategy	2	82.67	2.67	0.068
Lake(strategy)	3	31.22	5.71	0.001
Residual	17	5.47		
Total	22			
(d)　PUFA				
Strategy	2	0.16	7.53	0.061
Lake(strategy)	3	0.02	12.2	0.001
Residual	17	0.002		
Total	22			
(e)　SFA				
Strategy	2	0.01	1.46	0.344
Lake(strategy)	3	0.009	3.17	0.058
Residual	17	0.003		
Total	22			
(f)　SDA				
Strategy	2	0.10	249.3	0.001
Lake(strategy)	3	0.0004	0.283	0.279
Residual	17	0.0003		
Total	22			
(g)　SFA:PUFA				
Strategy	2	0.411	2.68	0.217
Lake(strategy)	3	0.154	2.31	0.107
Residual	17	0.067		
Total	22			
(h)　n3: n6				
Strategy	2	75.46	52.9	0.004
Lake(strategy)	3	1.43	1.21	0.308
Residual	17	1.18		
Total	22			
(i)　FA composition seston				
Strategy	2	29.52	1.01	0.522
Lake(strategy)	3	26.82	5.02	0.001
Residual	9	5.34		
Total	14			

Figure 1. Nonmetric multidimensional scaling (nMDS) plot of fatty acid (FA) methyl ester concentrations from lakes with *Daphnia* showing a) active (black symbols), b) diapause (white symbols) or c) both (grey symbols) overwintering strategies. Data points represent samples collected in fall from the six lakes (MS = Malla South, SA = Saanajärvi, SI = Simoncouche, LU = Lunzersee, BR = Big Rock, ME = Mekkojärvi).

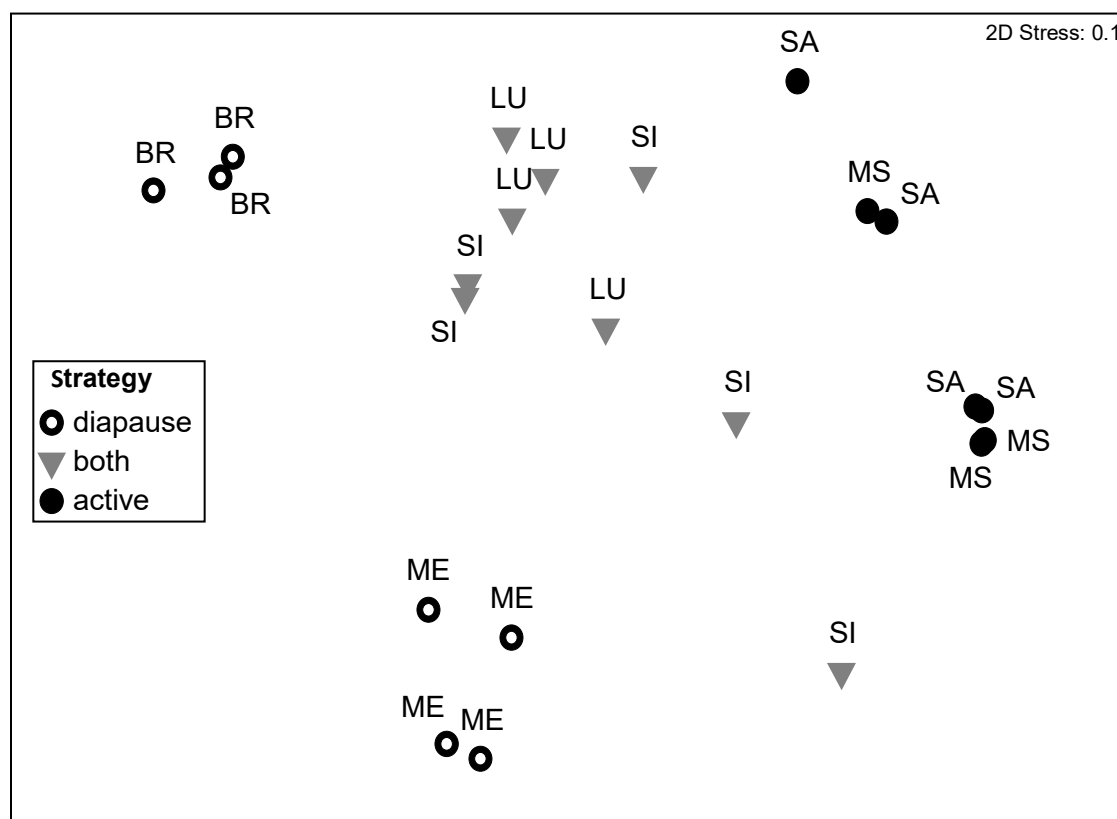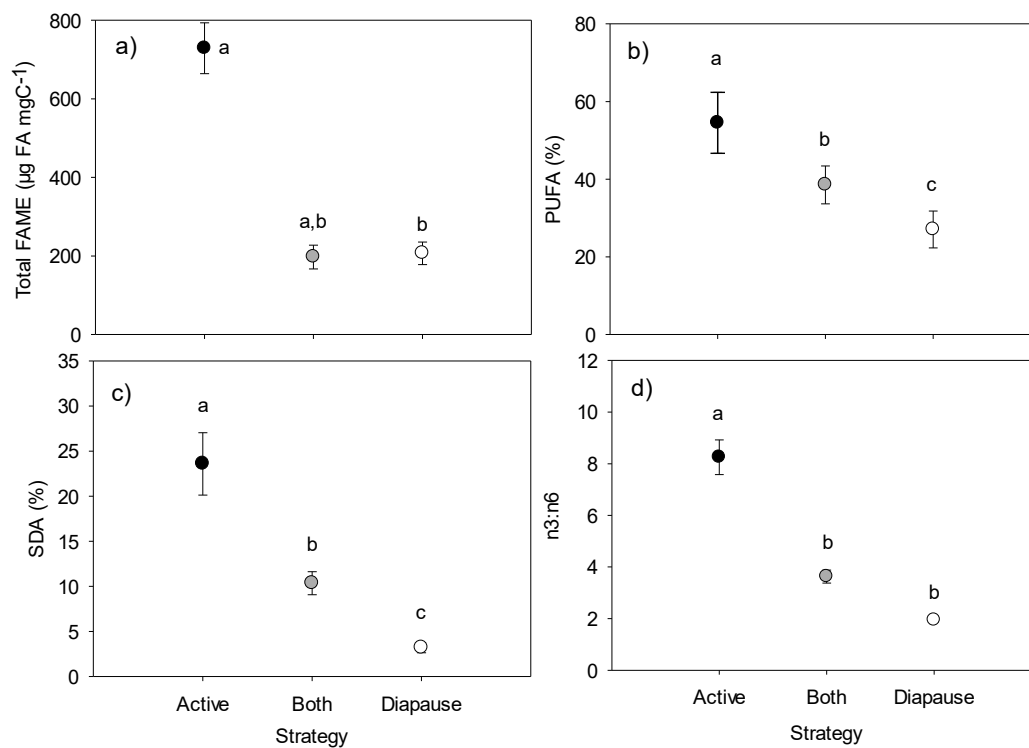Figure 2. *Daphnia* a) total FAME concentration (µg FA mgC^{-1}), b) percentage of PUFA of total FA, c) percentage of SDA (18:4n3) of total FA and d) n3:n6 ratio in communities showing active, diapause or both overwintering strategies at the onset of winter. Error bars are SE. Different letters indicate statistically different values.

Supplementary Information

SI Table 1. Seston a) total FAME concentration (μgFA mgC^{-1}), b) fatty acid composition (mole % of total fatty lipids) for saturated (ΣSFA), monounsaturated (ΣMUFA), and polyunsaturated (ΣPUFA) fatty acids, and c) ratios of ΣSFA:PUFA and Σn3:n6 during the fall. All values are mean ±SD, no value is shown when SD <0.1. n = number of replicates, nd = no data.

	Active		Both strategies		Diapause	
	Malla South	Saana-järvi	Simon-couche	Lunzersee	Big Rock	Mekkojärvi
	n=3	n=3	n=1	n=4	n=1	n=3
a) **ΣFAME**	**28.9 ±9,5**	**33.8 ±4,8**	**19.1**	**65.3 ±29**	**80.2**	**42.2 ±19**
b) **ΣSFA**	**67,2 ±0.3**	**48.4**	**43.8**	**41.3 ±0.05**	**47.9**	**88.6 ±0.1**
14:0	41±0.4	6.7	8.2	9.7	3.4	80.0
15:0	1,6	4.0	2.2	2.1	11.1	0.0
16:0	21.8 ±0.2	33.9	29.4	23.1	22.5	6.8
18:0	2.6	2.2	4.0	6.5	10.9	1.8
ΣMUFA	**24.5 ±0.2**	**44.0**	**31.9**	**27.1**	**39.2**	**7.5**
16:1n7	13.6	29.4	15.0	19.1	18.5	4.2
18:1n9	8,3	10.9	11.5	4.1	15.7	3.3
16:1n9	2.7	3.7	5.1	3.6	5.1	0.0
18:1n7	0.0	0.0	0.4	0.2	0.0	0.0
ΣPUFA	**8.3**	**7.6**	**24.3**	**31.5**	**12.9**	**3.9**
18:2n6	1.0	2.9	4.2	6.9	2.9	0.0
18:3n3	2.9	0.0	8.2	9.3	1.0	0.0
18:4n3	0.0	0.0	1.4	0.7	1.0	0.0
18:3n6	1.6	0.0	0.0	0.2	0.0	0.0
20:4n3	0.0	2.1	4.9	5.8	1.9	2.3
20:4n6	0.0	0.4	2.8	4.3	0.0	0.0
20:5n3	2.9	2.1	2.8	2.3	1.5	1.5
22:6n3	1.5	nd	nd	1.9	nd	0.0
c) **ΣSFA:PUFA**	**8.1 ±1.2**	**6.4 ±0.4**	**1.8**	**1.3 ±0.3**	**3.7**	**22.8±9.2**
Σn3:n6	**7.1 ±0.2**	**1.3 ±0.1**	**2.5**	**1.7**	**3.3**	**0.0**

SI Table 2. Site-specific average (±SD) values of *Daphnia* a) body fat (% of dry weight), b) total FAME concentration (µgFA mgC^{-1}), c) fatty acid composition (mole % of total fatty lipids) for saturated (ΣSFA), monounsaturated (ΣMUFA), and polyunsaturated (ΣPUFA) fatty acids, d) ratios of ΣSFA:PUFA and Σn3:n6 during the fall when *Daphnia* are either preparing to enter diapause or remain active during winter ice cover. Error values not shown when SD <0.1. n = number of replicates, nd = no data.

	Active		Both strategies		Diapause	
	Malla South	Saanajärvi	Simon-couche	Lunzer-see	Big Rock	Mekko-järvi
	D. umbra	*D. umbra*	*D. galeata,* *D. dubia,* *D. longiremis*	*D. longispina*	*D. longispina*	*D. longispina*
	n=3	n=4	n=5	n=4	n=3	n=4
a) Body fat	59.5 ±9.2	57.7 ±12.8	30.9 ±3.3	nd	23.9 ±5.3	41.2 ±11.1
b) ΣFAME	**838.9±83.7**	**646.3±181**	**258.4 ±65.6**	**121±49**	**270.2±43**	**159.2 ±58**
c) ΣSFA	**26.9**	**27.0**	**36.4 ± 0.1**	**27.8**	**30.4**	**22.6**
14:0	9.9	9.5 ±0.1	16.7 ± 0.1	6.5	2.1	5.3
15:0	1.0	0.8	0.7	0.9	1.0	1.1
16:0	10.5	10.9	15.2	15.9	19.1	10.7
17:0	0.2	0.2	0.6	0.9	0.8	0.7
18:0	5.3	5.8	3.2	3.5	7.4	4.7
ΣMUFA	**15.0**	**15.8**	**28.0**	**24.8**	**31.5**	**54.5**
16:1n7	5.9	5.8	9.2	8.6	11.4	39.3
18:1n9	5.3	5.9	9.1	8.0	11.5	4.8
16:1n9	1.3	1.4	6.5 ±0.1	1.4	0.7	1.1
18:1n7	2.5	2.7	3.2	6.8	7.9	9.4
ΣPUFA	**58.1**	**57.0**	**35.6 ±0.1**	**47.4**	**38.1**	**22.9**
18:2n6	3.3	3.7	4.2	4.7	6.6	3.3
18:3n3	5.0	5.3	5.0	8.7	10.2	3.4
18:4n3	26.0	25.4	10.4	12.7	2.7	4.3
18:3n6	1.2	1.3	0.9	0.6	0.6	0.6
20:4n3	10.2 ±0.1	7.8 ±0.1	0.5	0.7	0.5	0.7
20:4n6	1.5	1.7	3.7	3.9	5.4	4.0
20:5n3	10.3	10.9	10.2	15.3	11.9	6.1
22:6n3	0.6	0.9	0.7	0.9	0.2	0.5
d) ΣSFA:PUFA	**0.5**	**0.5 ±0.1**	**1.0 ±0.5**	**0.6 ±0.1**	**0.8**	**1.0 ±0.1**
Σn3:n6	**8.7 ±1.5**	**7.5 ±2.1**	**3.0 ±0.6**	**4.2 ±0.4**	**2.0 ± 0.1**	**1.9 ±0.2**